MEDICINE

Bispecific antibodies

Bispecific antibodies have emerged as molecules with a multitude of talents

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Bispecific antibodies (bsAbs) bind two different epitopes on the same or different antigens. Through this dual specificity for soluble or cell-surface antigens, bsAbs exert activities beyond those of natural antibodies, offering numerous opportunities for therapeutic applications. Although initially developed for retargeting T cells to tumors, with a first bsAb approved in 2009 (catumaxomab, withdrawn in 2017), exploring new modes of action opened the door to many additional applications beyond those of simply combining the activity of two different antibodies within one molecule. Examples include agonistic "assembly activities" that mimic the activity of natural ligands and cofactors (for example, factor VIII replacement in hemophilia A), inactivation of receptors or ligands, and delivery of payloads to cells or tissues or across biological barriers. Over the past years, the bsAb field transformed from early research to clinical applications and drugs. New developments offer a glimpse into the future promise of this exciting and rapidly progressing field.

Monoclonal antibodies (mAbs) comprise antigen-binding sites formed by the variable domains of the heavy and light chain and an Fc region that mediates immune responses. BsAbs, produced through genetic engineering, combine the antigen-binding sites of two different antibodies within one molecule, with a plethora of formats available (*1*). Conceptually, one can discriminate between bsAbs with combinatorial modes of action where the antigen-binding sites act independently from each other, and bsAbs with obligate modes of action where activity needs binding of both, either in a sequential (temporal) way or dependent on the physical (spatial) linkage of both (see the figure) (*2*). BsAbs approved as drugs are so far in the obligate dual-binding category: A T cell recruiter (blinatumomab) against cancer and a factor VIIIa mimetic to treat hemophilia A (emicizumab). Most but not all of the more than 100 bsAbs in clinical development address cancers. Some are in late stage (such as amivantamab, epcoritamab, faricimab, and KNO46), but most are still in early stages (*2*). Most of these entities enable effector cell retargeting to induce target cell destruction.

An increasing number of programs also explore alternative modes of action. This includes bsAbs that target pathways involved in tumor proliferation (such as amivantamab), invasion, ocular angiogenesis (such as faricimab), or immune regulation by blocking receptors and/or ligands, mainly in a combinatorial manner. Challenges for all of these entities are potential adverse effects, toxicity in normal tissues, and overshooting and systemic immune responses, especially with T cell retargeting or immune-modulating or activating entities. Such issues need to be carefully addressed.

Most of the bispecific T cell engagers comprise a binding site for a tumor-associated antigen and CD3 [a component of the T cell receptor (TCR) activation complex] as trigger molecule on T cells. To prevent or ameliorate "on-target, off-tumor" effects of T cell recruiters, approaches currently investigated include the modulation of target affinities and mechanisms to allow conditional activation upon target cell binding. Thus, a reduced affinity for CD3 increased tolerability by reducing peripheral cytokine concentrations that are associated with nonspecific or overshooting immune reactions (*3*). Similarly, reduced affinity for the target antigen was shown to ameliorate cytokine release and damage of target-expressing tissues (*4*). Tumor selectivity can be further increased by implementing avidity effects—for example, by using 2+1 bsAb formats with two low-affinity binding sites for target antigens and monovalent binding to CD3 (*4*).

In further approaches, binders to CD3 were identified that efficiently trigger target cell destruction without inducing undesired release of cytokines, demonstrating the importance of epitope specificity to potentially uncouple efficacy from cytokine release (*5*). Complementing these T cell–recruiting principles, the nonclassical T cell subset of γ9δ2 T cells with strong cytotoxic activity emerged as potent effectors, which can be retargeted with bsAbs binding to the γ9δ2 TCR. Thereby, global activation of all T cells, including inhibitory regulatory T cells (T_{reg} cells), through CD3 binding, may be avoided (*6*). However, even these approaches might result in a narrow therapeutic window to treat solid tumors because of T cell activation in normal tissues.

Consequently, there are several approaches to conditionally activate T cells within tumors, including a local liberation of the CD3-binding sites or triggering local assembly of CD3-binding sites from two half-molecules. For example, CD3-binding sites have been masked by fusing antigen binding or blocking moieties—such as peptides, aptamers, or anti-idiotypic antibody fragments—to one or both variable domains. These moieties are released within the tumor by tumor-associated proteases, or through biochemical responses to hypoxia or low pH (*7*). This approach can also be applied to confer specific binding of antibody therapeutics, including bsAbs, to antigens on tumor cells (*8*).

An on-target restoration of CD3-binding sites requires application of two target-binding entities, each comprising parts of the CD3-binding site, which assemble into functional binding sites upon close binding of both half-antibodies. The feasibility of this approach was recently shown, for example, for a split T cell–engaging antibody derivative (Hemibody) that targets a cell surface antigen (*9*). Such approaches can also be applied to half-antibodies that recognize two different targets expressed on the same cell, further increasing tumor selectivity.

Regarding T cell engagers, increasing efforts are made to target not only cell-surface antigens expressed on tumor cells but also human leukocyte antigen (HLA)–presented tumor-specific peptides. This expands the target space of bsAbs toward tumor-specific intracellular antigens and can be achieved by using either recombinant TCRs or antibodies with TCR-like specificities combined with, for example, CD3-binding arms to engage T cell responses. A first TCR–anti-CD3 bispecific molecule is in phase I and II trials to treat metastatic melanoma (*10*). A challenge of this approach is the identification of TCRs or TCR-like antibodies that bind the peptide in the context of HLA with high affinity and specificity, without cross-reacting with related peptides to reduce or avoid off-target activities. Comprehensive screening tools and implementation of computational approaches are being developed to achieve this task.

A rapidly growing area of bsAbs in cancer therapy is their use to foster antitumor immune responses. Here, they are especially applied for dual inhibition of checkpoints that prevent immune responses—for example, programmed cell death protein 1 (PD-1) and its ligand (PD-L1), cytotoxic T lymphocyte–associated antigen 4 (CTLA-4), or lymphocyte activation gene 3 (LAG-3; for example,

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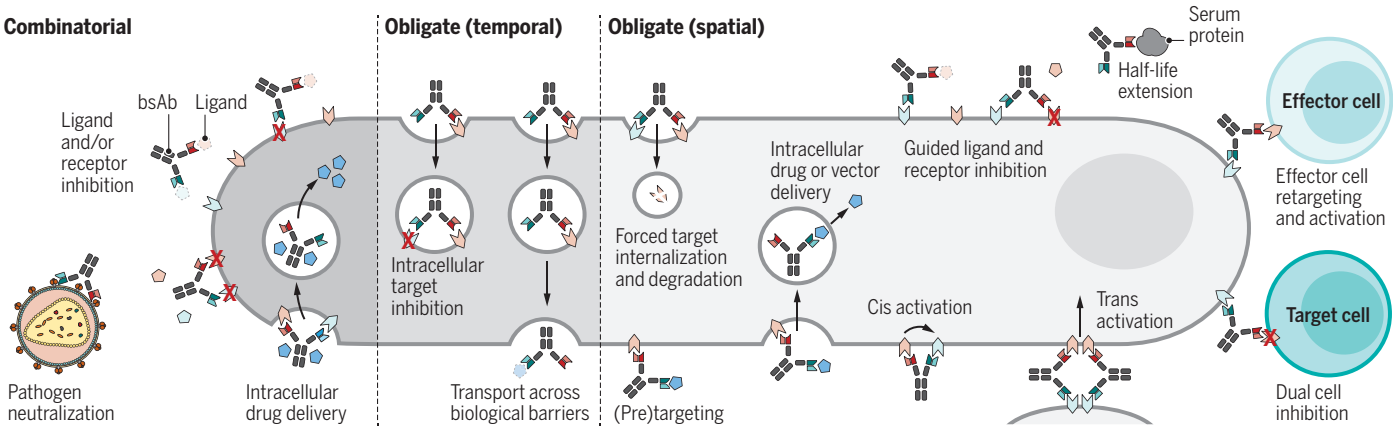
Modes of action of bispecific antibodies

There are >100 bispecific antibodies (bsAbs) in clinical development. These are broadly classified as combinatorial, combining the activity of two antibodies within one molecule, or obligate, where combining both binding sites creates a temporal or spatial activity.



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KNO46). Tumor-targeted bsAbs can also target costimulatory factors such as CD28 or 4-1BB ligand (4-1BBL) to enhance T cell responses when combined with PD-1 blockade or to provide an activity-enhancing costimulatory signal in combination with CD3-based bsAbs (*11*). Furthermore, bsAbs are being developed for local effects by targeting one arm to antigens that are expressed by tumor cells or cells of the tumor microenvironment (*2*).

Clinical application of bsAbs now expands to other therapeutic areas, including chronic inflammatory, autoimmune, and neurodegenerative diseases; vascular, ocular, and hematologic disorders; and infections. In contrast to mAbs, bsAbs can inactivate the signaling of different cytokines with one molecule to treat inflammatory diseases (*12*). Simultaneous dual-target binding is not essential to elicit activity for bsAbs against combinations of proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin-1α (IL-1α), IL-1β, IL-4, IL-13, IL-17, inducible T cell costimulator ligand (ICOSL), or B cell–activating factor (BAFF). This presumably also applies to blockade of immune cell receptors, although dual targeting might confer increased efficacy due to avidity effects and increased selectivity through simultaneous binding of two different receptors.

A further application of combinatorial dual targeting is in ophthalmology. Loss of vision in wet age-related macular degeneration (AMD) results from abnormal proliferation and leakiness of blood vessels in the macula. This can be treated with antibodies that bind and inactivate factors that stimulate their proliferation (*13*). In contrast to mAbs or fragments that recognize individual factors, bsAbs bind two such factors. For example, faricimab that binds vascular endothelial growth factor A (VEGF-A) and angiopoietin-2 (ANG2), demonstrated dual efficacy in preclinical studies, and is currently in phase 3 trials.

BsAbs with obligate modes of action that mandate simultaneous dual-target binding are "assemblers" that replace the function of factors necessary to form functional protein complexes. One of these bsAbs with an assembly role (emicizumab, approved in 2018) replaces factor VIIIa in the clotting cascade. Deficiency of factor VIII causes hemophilia A, which can be overcome by substitution with recombinant factor VIII. However, a proportion of patients develop factor VIII–neutralizing immune responses and no longer respond to therapy. To overcome this, a bsAb was developed with binding sites that recognize and physically connect factors IXa and X, a process normally mediated by factor VIIIa. Extensive screening of a large set of bsAbs was required to identify those that combine suitable epitopes with optimized affinities and geometry to serve as functional factor VIIIa mimetics (*14*). This exemplifies the complexity of identifying the best bsAb for therapeutic applications.

A mode of action requiring sequential binding of two targets is the transport of bsAbs across the blood-brain barrier (BBB). This is a tight barrier of brain capillary endothelial cells that controls the transport of substances between the blood and the cerebrospinal fluid—the brain parenchyma. Passage of large molecules, including antibodies, across the BBB is thereby restricted. Some proteins, such as transferrin or insulin, pass through the BBB by way of transporters on endothelial cells. Antibodies that bind these shuttle molecules, such as the transferrin receptor (TfR), can hitchhike across the BBB. BsAbs that recognize brain targets (such as β-amyloid for Alzheimer's disease) and TfR with optimized affinities, epitopes, and formats can thereby enter the brain. Such bsAbs are currently in clinical evaluation to treat neurodegenerative diseases (*15*).

In the past years, there has been a transition from a technology-driven phase, solving hurdles to generate bsAbs with defined composition, toward exploring and extending the modes of action for new therapeutic options. The challenge of generating bsAbs is not only to identify suitable antigen pairs to be targeted in a combined manner. It is now recognized that the molecular composition has a profound impact on bsAb functionality (*13*). That more than 30 different bsAb formats are in clinical trials proves that development is now driven by a "fit for purpose" or "format defines function" rationale. Many candidates differ in their composition, affecting valency, geometry, flexibility, size, and half-life (*1*). Not all members of this "zoo of bsAb formats" qualify to become drugs. Strong emphasis is therefore on identifying candidates that exhibit drug-like properties and fulfill safety, developability, and manufacturability criteria. There is likely to be an exciting new wave of bsAb therapeutics available in the coming years. ■

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